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Research report

The aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) reduces disinhibitory behavior in intact adult male rats treated with a high dose of testosterone

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ABSTRACT

Anabolic androgenic steroids and high testosterone doses have been reported to induce impulsive behavior in man and behavioral disinhibition in rats. The purpose of the present study was to investigate whether aromatization of testosterone to estradiol is of importance for the behavioral disinhibiting effect of a high testosterone dose in adult male rats. Testosterone administered via five testosterone-filled silastic capsules implanted subcutaneously (s.c.) to non-castrated, group-housed rats for six days induced behavioral disinhibition in a modified Vogel's drinking conflict model and yielded supraphysiological serum levels of testosterone and increased accessory sex organ weights. Moreover, concurrent administration of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD; 60 mg/kg/day s.c.) decreased behavioral disinhibition in testosterone-treated rats (without affecting accessory sex organ weights) while behavior was not significantly affected in sham-treated animals. Since some reports indicate that ATD, in addition to inhibit aromatase, also may affect the binding of testosterone to the androgen receptor, the effect of the non-steroidal androgen receptor antagonist flutamide was investigated. Flutamide treatment did not affect disinhibited behavior in testosterone-treated rats. However, in sham-treated animals, flutamide (50 mg/kg/day) produced behavioral disinhibition. These results suggest that estradiol is of importance in the mechanisms underlying behavioral disinhibition in non-castrated rats treated with a high testosterone dose. Speculatively, aromatization may be involved in pro-impulsive effects of high testosterone doses in humans.

1. Introduction

Clinical studies and case reports indicate that abuse of anabolic androgenic steroids (AAS) and administration of high testosterone doses induce impulsive and aggressive behavior [9,20,22,37,39–41]. Among abusers, the actual dosage of AAS is generally unknown since illegal steroids frequently are incorrectly labeled [7]. The mechanisms underlying the pro-impulsive effect of high testosterone and AAS doses in man are not known. Further support for a relation between testosterone and impulsivity has been gained from a study showing that patients suffering from bulimia nervosa, a disorder at least to some extent characterized by poor impulse control, display higher serum levels of free testosterone compared with age-matched controls [48]. However, a lack of correlation between physiological testosterone levels in cerebrospinal fluid (CSF) and impulsive behavior has been reported in humans [60,61] and nonhuman primates [23], and these authors instead suggest that CSF testosterone levels correlate to aggressive, rather than impulsive, behavior.

In intact male rats, the AAS nandrolone induces disinhibitory behavior in the elevated plus-maze [27]. Moreover, high doses of testosterone also induce such a behavior in the elevated plus-maze [4] and in Vogel's conflict model [3,51]. Castration, on the other hand, enhances shock-induced behavioral inhibition in Vogel's conflict test in group-housed adult rats and testosterone substitution prevents this effect [50]. However, castration does not affect this behavior in single-housed adult rats [49].

Conflict models have been widely used to study anxiety. However, disinhibited behavior, such as that seen in Vogel's conflict model, might instead reflect impulsive behavior, at least after certain pharmacological manipulations. Brain serotonin depletion of rats induces behavioral disinhibition in conflict models [reviewed in [46]], but clinical findings do not support the assumption that decreased serotonin synthesis leads to anxiolysis [16]. Instead, several investigators have found an association between low concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid in CSF and impulsivity in man [e.g. [28]]. Moreover, high doses of testosterone induce disinhibited behavior in rats, but high doses

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of androgens are not anxiolytic in man. Instead, AAS use is associated with decreased impulse control [9,20,39–41] and anxiety [38]. In a review, Soubrié [46] argued that anticonflict behavior may be interpreted in terms of decreased inhibitory control.

Thus, most studies indicate that treatment with high doses of testosterone promotes disinhibitory behavior in rats and impulsive behavior in man. However, little is known about underlying mechanisms. Testosterone can either be aromatized to estradiol by the enzyme aromatase or converted to dihydrotestosterone by 5alpha-reductase [42]. Estradiol stimulates estradiol receptors alpha and beta [45], whereas testosterone and dihydrotestosterone stimulate androgen receptors [42]. Moreover, dihydrotestosterone can be metabolized to 3alpha-diol (5α-androstane-3α, 17β-diol) by the enzyme 3alpha-hydroxysteroid dehydrogenase, and 3alpha-diol is then converted to androsterone (5α-androstane-3α-ol-17-one) by the action of 17β-hydroxysteroid dehydrogenase [24]. Both 3alpha-diol [21] and androsterone [57] are positive modulators at the $GABA_A$ receptor. 3alpha-diol induces behavioral disinhibition both in the elevated plus-maze and in the Vogel's conflict model [18], as do various other positive modulators at the $GABA_A$ receptor [52]. Estradiol also produces disinhibited behavior, presumably by stimulating estradiol receptor beta [26,29,58]. Thus far, no study has been published investigating whether estradiol is involved in mechanisms underlying behavioral disinhibition in intact male rats treated with high doses of testosterone.

The main objective of this study was to examine effects of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) on behavioral disinhibition displayed by adult male rats treated with a high testosterone dose. In order to resemble the clinical situation, intact rats were used. ATD was used since it has been reported that ATD lacks androgenic, antiandrogenic, estrogenic or antiestrogenic activity [5,6] and does not affect serum levels of luteinizing hormone in castrated or intact monkeys [15]. Also there is evidence that ATD induces behavioral effects and thus penetrates the blood–brain-barrier [47,55]. However, some researchers suggest that ATD, in addition to inhibit aromatase, also affects the binding of testosterone to the androgen receptor [25]. In order to control for such a possible unspecific effect of ATD, behavioral effects of the non-steroidal androgen receptor antagonist flutamide were examined in the present experiments and accessory sex organ weights were estimated in ATD-treated rats.

2. Materials and methods

2.1. Animals

A total of 290 male Wistar rats (Taconic, Ry, Denmark) weighing 270–360 g (8–10 weeks old) were used. The animals were kept under controlled light–dark conditions (light on at 7:00 a.m. and off at 7:00 p.m.) and at constant cage temperature (20 °C) and cage humidity (40–50%). A minimum adaptation period of seven days was allowed to the animal maintenance facilities of the department prior to the start of the experiments. The rats, housed four to each cage (55 cm × 35 cm × 20 cm), had free access to water and food (rat standard feed, Harlan Teklad Europe, UK) except during experiments. All experiments were approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden and conducted in a manner complying with the European Community guidelines for the use of experimental animals.

2.2. Capsule implantation

Silastic capsules (effective length: 40 mm, inner diameter: 1.57 mm, see Section 2.7. below) were filled with crystalline testosterone. Capsules were incubated in 0.9% NaCl for 24 h. They were washed first in 70% ethanol for 30 min and then in saline for 30 min prior to implantation. The rats were anaesthetized with isoflurane (3.5–4.0% in air) and five silastic capsules containing testosterone or five empty capsules were implanted subcutaneously in the back (see Section 2.7). Behavioral experiments were initiated six days after capsule implantation.

2.3. Shock-induced behavioral inhibition

A modified Vogel's drinking conflict model was used [52]. On the first day of the experiment, the animals were adapted for 20 min to a Plexiglas box (inner-dimensions 30 cm × 24 cm × 20 cm) enclosed in a soundproof cage and equipped with a grid-floor of stainless steel bars and a drinking bottle containing a 5.5% (w/v) glucose solution. A 24-h period of water deprivation then followed. Thereafter, animals were adapted to the test-chamber again for a further 20 min. Again, there was free access to the glucose solution. After a further 24 h of water deprivation, the animals were returned to the Plexiglas box. When approaching the drinking-spout (usually within 20 s) the animal was allowed to drink for 30 s, after which the first electric shock (0.16 mA for 2 s) was administered between the spout of the drinking bottle and the grid-floor. An electric shock was administered upon each further attempt to drink. The number of shocks accepted during a 10-min session was recorded. All experiments were carried out between 9 A.M. and 5 P.M. Animals were tested only once.

2.4. Shock threshold and drinking motivation tests

In order to obtain conformity with Vogel's conflict test the animals were treated identically in both the shock threshold and drinking motivation tests [52] including water deprivation. Each rat was then placed in the Plexiglas box previously described. The shock threshold was determined step-wise by manually increasing the current delivered through the grid-floor (0.06, 0.08, 0.10, 0.13, 0.16, 0.20, 0.25 mA) until the rat showed an avoidance reaction to the electrical stimulus (jump, jerk or similar) as judged by an assistant that was blind to the treatment and the shock level applied. There was a 15-s shock-free interval between each step. The current amplitude threshold was recorded. Immediately after the shock threshold had been determined (see above), each rat was placed in an individual cage with a drinking bottle containing approximately 50 ml of a 5.5% (w/v) glucose solution. The total amount of liquid (g) consumed during 10 min was recorded for each rat.

2.5. Experimental designs

It has earlier been shown that five, but not three or one, silastic capsules (with same dimensions as used here) filled with testosterone implanted subcutaneously to intact male rats for six days induced behavioral disinhibition and produced approximately nine times higher serum levels of testosterone compared with controls [51]. Interestingly, high doses of testosterone administered to humans, producing aggressive behavior [56], give rise to a more than six-fold increase in serum levels of testosterone and the authors believed that even higher testosterone doses are commonly used by AAS abusers [2]. The present experiments were designed in order to be similar to the situation in humans taking high doses of testosterone. Therefore, in order to resemble the clinical situation, testosterone was administered to intact rats through five testosterone-filled capsules.

2.5.1. Experiment 1

The rats ($n = 81$) were randomized to six treatment groups. Three groups received sham treatment (sham) and three groups received testosterone (test). The animals were injected subcutaneously with vehicle (veh: 10% ethanol in propylene glycol), ATD 30 mg/kg (2 ml/kg) or ATD 60 mg/kg (2 ml/kg) and two independent experiments were performed in each treatment group. Thus, the following treatment groups were generated: (a) sham/veh, $n = 10$; (b) sham/ATD30, $n = 16$; (c) sham/ATD60, $n = 15$; (d) test/veh, $n = 9$; (e) test/ATD30, $n = 15$ and (f) test/ATD60, $n = 16$. The ATD doses used here, 30 and 60 mg/kg, were chosen since earlier studies showed behavioral effects of that dose span on, e.g., sexual behavior [25].

Rats were administered either ATD (30 or 60 mg/kg) or vehicle 28 h and then again four hours prior to capsule implantation. After implantation, they received daily injections of vehicle or ATD for the next six days. Then, four hours after the last injection, rats were tested with respect to shock-induced behavioral inhibition. On the following day, ATD was given to five to seven rats from each treatment group (38 out of 81 rats mentioned above). They were sacrificed four hours later by decapitation and accessory sex organs were weighed.

Another set of animals ($n = 56$) was tested with respect to shock thresholds and drinking motivation in the above-described manner.

2.5.2. Experiment 2

The rats ($n = 63$) were divided in the same way as in Experiment 1 but were instead injected subcutaneously with vehicle (veh: 10% ethanol in propylene glycol), flutamide (flu) 25 mg/kg (2 ml/kg) or flutamide 50 mg/kg (2 ml/kg) yielding six treatment groups: (a) sham/veh, $n = 12$; (b) sham/flu25, $n = 9$; (c) sham/flu50, $n = 9$; (d) test/veh, $n = 14$; (e) test/flu25, $n = 9$ and (f) test/flu50, $n = 10$. The flutamide doses in the present experiment, 25 and 50 mg/kg, were used on the basis of earlier studies [17,53].

Rats were administered either flutamide (25 or 50 mg/kg) or vehicle 28 h and then again four hours prior to capsule implantation. After implantation, they received injections of vehicle or flutamide daily for the next six days. Then, four hours after the last injection, rats were tested with respect to shock-induced behavioral inhibition. On the following day, flutamide was given to seven randomly chosen rats from each treatment group (42 out of 63 rats mentioned above). They were sacrificed four hours later by decapitation and accessory sex organs were weighed.

Another set of animals ($n = 36$) was tested with respect to shock thresholds and drinking motivation in the above-described manner.